

# Cyclosporin Trough Concentration And Its Relationship

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## Abstract

**Background-** Managing maintenance cyclosporin regimens after transplantation requires careful attention to efficacy, dosing and graft adverse effects. The aim of this study was to investigate the relationship between cyclosporin trough concentration and graft rejection after kidney transplantation.

**Methods-** Data were collected from sixty five stable patients (47 M: 18 F) of mean age 36 years (range 18 to 60 years) and mean weight of 55 kg (range 40 to 75 kg). Multivariate analysis correlated laboratory values and clinical parameters including: donor source, kidney preservation time, human leukocyte antigen match, immunosuppressive regimen and weight-adjusted maintenance cyclosporin with the occurrence of graft rejection.

**Results-** The mean value of cyclosporin whole blood levels was reflected by 36.6-fold variability (range 30-1098 µg/l) in the study population. Lower values of C0 were correlated with an increased occurrence of graft rejection. The mean trough blood level (178 versus 265 ug/l;  $P = 0.032$ ) and the mean daily orally dosage of cyclosporin (4.7 versus 6.3 mg/kg/body weight;  $P = 0.010$ ) in rejected recipients (56%) was significantly lower than non-rejected recipients. Significance correlations existed in the rejected recipients ( $n=36$ ) between C0 and creatinine ( $r = -2.7$ ,  $P = 0.006$ ), serum potassium ( $r = -1.9$ ,  $P = 0.055$ ), alkaline phosphatase ( $r = -1.89$ ,  $P = 0.05$ ). These associations might reflect the liver and renal dysfunction prevailed around the time of rejection.

**Conclusions-** Optimizing cyclosporin level reasonably and preventing graft rejection seem to be the major factor improving patient quality of life after kidney transplantation in Isfahan/Iran.

## INTRODUCTION

The administration of cyclosporin to organ transplant recipients is associated with marked pharmacokinetic variability [1]. These inter-individual variations may influence the immune response and susceptibility to drug toxicity in this population [2,3]. Recipients of transplanted organ require careful follow-up in both the early and late post-transplant periods [4]. Monitoring should focus on graft function [5] and the most common complications of immunosuppression therapy [6]. Extreme inter and intra individual variability in absorption of cyclosporin, has complicated the clinical use of this agent after transplant [7]. Distribution is affected by the lipoprotein concentration in plasma and by the haematocrit [8]. Heterogeneity in gut cytochrome P-450 3A4 gene expression also explains some of the wide variability in cyclosporin kinetics. In addition, drugs that induce or inhibit CYP3A4 influence the CYP3A4-dependent metabolism [9,10]. Demographic factors such as

age, gender, and race may also contribute to the variability of cyclosporin pharmacokinetics [11]. Among renal allograft recipients, there is a considerable variability in cyclosporin trough levels. Previous studies suggested that, the selected initial dose of cyclosporin (9.2 mg/kg/day) reflected target blood level of 150-400 ug/l (0-90 days) and 100-300 ug/l (>90 days) [12,13]. Therefore, maintaining cyclosporin concentration in the narrow therapeutic window between graft rejection and toxic side effects requires a structured program of blood level monitoring. This objective has been assisted in by trough concentration measurements of the cyclosporin, i.e., blood samples are drawn as close as possible to the time of the next oral dose (C0) in the city of Isfahan/Iran. In the presence of low blood trough concentration rejection of transplanted organ is reasonably possible [14].

Rejection can be defined as graft damage arising from

response to the transplanted organ by the recipient's immune system. The two major presentations after transplantation are acute and chronic rejection with hyperacute rejection rarely encountered [15].

Acute rejections may occur at any time after grafting with the first episodes usually occurring around the 7th day. The diagnosis, suggested by clinical signs and biochemical abnormalities. It is confirmed by ultrasound and biopsy [16]. Chronic rejection, which can present as early as the first two weeks after liver transplantation, is characterised by slowly declining graft function and is usually accompanied by the corresponding elevation of liver enzymes and especially bilirubin [17]. In kidney transplant recipients biochemical variables confirm increasing in creatinine and blood urinary nitrogen (BUN) [15,18].

To decrease the incidence of rejection associated with under administrations, the best solution to date has been the simple adjusting of dosage regimens on the basis of trough blood levels. The aim of this study was to investigate the relationship between cyclosporin trough concentration and graft rejection in stable kidney transplant recipients.

## SUBJECTS AND METHODS

A retrospective, randomized study was undertaken in Isfahan/Iran to investigate episodes of rejection after kidney transplantation.

All patients received cyclosporin twice daily as immunosuppressive maintenance treatment. Cyclosporin blood levels were measured by the radioimmunoassay technique using monoclonal antibodies. The calibration range of the method was 10-1380 ug/l. Sensitivity was about 10 ug/l.

Initially statistical analysis of 3.5 years of cyclosporin pre-dose blood levels was performed on 1847 laboratory values obtained from 250 patients (89 F: 161 M) with a mean age of 42 years (range: 10-72 years) and minimum weight of 64 kg (range: 48-100 kg). Because of wide variability in clinical parameters, patients were required to meet the following inclusion criteria of: alive and first transplanted recipients, an age between 18 to 65 years, a body weight between 40 and 90 kg, a minimum of three weeks after transplant, no changes of cyclosporin dosage for at least three weeks and no co-medication with drugs which are known to influence metabolism.

Therefore sixty five kidney recipients (comprised of: 47

males and 18 females) of mean age 36 years (range; 20 to 60 years) and mean weight of 55 kg (range; 40 to 75 kg) was further studied. Baseline demographic characteristics of the patients are shown in Table 1. Drug exposure was estimated by the average cyclosporin concentration (n=400). Clinical parameters including: (clinical biochemistry and haematology results, weight-adjusted maintenance cyclosporin dose, other drugs, date of transplant, human leukocyte antigen match, donor source, kidney preservation time, occurrence of graft rejection) were also recorded in Excel. Local ethical committee approved the study. Acute allograft rejection was suspected by the presence of clinical signs, such as increased serum creatinine, decreased urine output or graft tenderness. The diagnosis of acute rejection was biopsy confirmed in most cases. Chronic rejection was identified by clinical and biopsy criteria.

**Figure 1**

Table 1: Baseline demographic and pharmacokinetic characteristics of patients after kidney transplantation.

	Gender (F: M)	Age (yr)	Weight (kg)	C <sub>0</sub> (ug/l)	Dose (mg/kg)
<b>Total Group</b>	18: 47	36	55	258.7	8.5
<b>(n= 65)</b>		(20-60)	(40-75)	(30-1000)	(3.4-9.5)
<b>Rejected</b>	11: 25	33	58	178	4.7
<b>Recipients (36)</b>		(25-60)	(40-68)	(39-1000)	(3.4-7.8)
<b>Non-Rejected</b>	7: 22	42	60	265	6.3
<b>Recipients (29)</b>		(20-58)	(48-75)	(30-856)	(4.8-9.5)
<b>P values</b>	0.222	0.132	0.259	*0.01	*0.03

Abbreviations: C<sub>0</sub>, Cyclosporin pre-dose level. Results are mean (minimum-maximum). Correlation coefficients were derived by Spearman Rank order analysis. P values were derived by Mann-Whitney U-test.

Comparisons between independent groups of data (rejected and non-rejected recipients) were made using the non-parametric Mann-Whitney U (ranking) test. Regression models (step wise) were used to examine the association between the dependent (C<sub>0</sub>, dose) and independent variables (clinical parameters). Bivariate correlations between two variables were derived using the Spearman Rank order correlation coefficient. A p value of less than 0.05 was considered statistically significant.

## RESULTS

Initial analysis of cyclosporin whole blood trough concentrations was reflected by 36.6-fold range of C<sub>0</sub> (266 ug/l; range: 30-1098 ug/l) in the study population (n= 1847), indicating some evidence (53% C<sub>0</sub><200 ug/l) to support the

hypothesis that low levels of cyclosporin identifies the occurrence of graft rejection in kidney transplant recipients. In a further step to reduce the variability in clinical parameters based on our target range (200-300 ug/l) [19], the mean cyclosporin C0 data sets (n = 400) obtained from sixty five stable kidney recipients (range: 30-1000 ug/l) were divided into four quartiles of mean C0 ranges (<100 ug/l, n=11; 100-200 ug/l, n= 10; 200-300 ug/l, n= 37 and greater than 300 ug/l n = 7) (Figure 1). However in none of the quartiles was there a trend towards freedom from the occurrence of rejection, but 56% of the kidney transplant recipients experienced episodes of rejection (mean time of graft rejection; 258 days, range: 30 days to 8 years) (Figure 2).

It is well known that, the variability in cyclosporin exposure obfuscates the relationship between therapeutic outcome and administered dose, thereby impeding the development of secure algorithm for cyclosporin. Therefore, regression models correlated demographic factors, laboratory values and clinical parameters with the occurrence of graft rejection. Patients' weight showed no correlation with C0 ( $r = 0.132$ ,  $P = 0.259$ ) and dose (mg/kg/day) ( $r = -0.168$ ,  $P = 0.311$ ) respectively. Analysis of pharmacokinetic variables including: C0 ( $r = 0.115$ ,  $P = 0.222$ ) and dose (mg/kg/day) ( $r = 0.189$ ,  $P = 0.145$ ) by gender showed no significant differences in the entire group of sixty five patients. The purpose of this study was to determine if we could target C0 range between 200-300 ug/l in kidney transplant recipients with out compromising safety. Whereas values lower than 200 ug/l in most cases was correlated with an increased occurrence of graft rejection. The mean trough blood level in the rejected recipients was 178 ug/l (range: 39 –1000 ug/l). In the 45 % of rejected kidney recipient's cyclosporin concentration was less than 200 ug/l. High values of cyclosporin in the rejected kidney recipients might demonstrate the large amounts of cyclosporin metabolites (with low immunosuppressive activity) that can cross-react with immunoassay methods [20, 4].

The mean cyclosporin trough blood level in the rejected kidney recipients (n=36) was 33% lower than non-rejected recipients (178 versus 265 ug/l) ( $P = 0.030$ ) (Mann-Whitney U-test). However we achieved target levels by given approximately 8.5 mg/kg/day in most cases (range; 3.4- 9.5 mg/kg/day) but dosage regimen was varied within different groups of rejected and non-rejected recipients. The mean daily orally dosage of cyclosporin (mg/kg), in the rejected recipients was significantly lower ( $P = 0.010$ ) than non-

rejected recipients (4.7 versus 6.3 mg/kg/body weight) (Figure 3).

Another major observation was the emergence of correlations of C0 with the result of some biochemical and haematology variables measured in the study population (Multiple regression analysis). In the rejected recipients abnormal biochemical and haematological results reflect the liver and renal dysfunction prevailing around the time of rejection episodes. The possible relationship to cyclosporin exposure was investigated by examining the correlations of clinical biochemistry and haematology parameters with cyclosporin C0. In the total group exposure to cyclosporin was correlated with serum potassium ( $r = 0.201$ ,  $P = 0.009$ ) (Figure 4). The occurrence of graft rejection markedly influenced clinical biochemistry results, particularly, creatinine (185 vs. 112;  $P = 0.004$ ), serum potassium (4.3 vs. 4.0;  $P = 0.044$ ) and alkaline phosphatase (80 vs. 40;  $P = 0.051$ ) that were all significantly higher in rejected recipients. Significance correlations existed in the rejected recipients (n=36) between C0 and creatinine ( $r = -2.7$ ,  $P = 0.006$ ), serum potassium ( $r = -1.6$ ,  $P = 0.035$ ) and alkaline phosphatase ( $r = -1.89$ ,  $P = 0.053$ ).

There were no correlations between clinical parameters (human leukocyte antigen match, donor source, kidney preservation time, other drugs) with the occurrence of graft rejection in this study population.

## DISCUSSION

After kidney transplantation optimization of cyclosporin level within a range that minimizes both the risk of rejection and drug toxicity is of great importance [21]. Initial analysis of 1847 cyclosporin pre-dose blood levels showed that in the 53%, laboratory values were lower than 200 ug/l. The wide variability in cyclosporin trough blood levels observed might be related to the amount of different metabolites produced that was varied between individual depending on their profile of metabolic enzymes [4, 5].

Because of wide variability in clinical parameters of patients (n=250), sixty five kidney transplant recipients were further assessed for the blood concentration-graft rejection relationship. To date, there is another study in which the influence of trough concentrations on renal allograft rejection was investigated. In several studies, a correlation between cyclosporin trough levels and the occurrence of rejection episodes was found [22,23]. Min et al studied the utility of cyclosporin trough concentrations as a monitoring tool for acute graft rejections after kidney transplantation.

They suggested that a cyclosporin concentration exceeding approximately 182 ug/l is essential to prevent graft rejection [12]. In our study out of sixty five patients, 56% experienced graft rejection (mean 258 days; range 30 days to 8 years). Previous studies showed that in cyclosporin treated patients the incidence of sub clinical rejection, which has been suggested as a cause of chronic allograft rejection, is reported to be approximately 30% [24]. Studies performed by Gjerston showed that delayed graft function and late acute rejection reduce graft half-lives by 30% and 50% [23].

As demonstrated by Oellerich et al. [19], there is a substantial variability among transplant centers concerning the therapeutic cyclosporin ranges for kidney transplantation. Based on our cyclosporin pre-dose target range of 200-300 ug/l in stable kidney recipients, the major concentration-related difference between the rejected and non-rejected recipients was a significant impact for the occurrence of graft rejection. In patients with the mean cyclosporin levels of 178 ug/l (range: 30 –1000 ug/l), graft rejection was increased as compared to patients with the mean levels of 265 ug/l (range: 100-800 ug/l). The wide variability in blood levels of cyclosporin might be related to the excretion (and therefore persistence) of different metabolites and the amount produced between individual recipients [4, 5]. The mean dose (mg/kg) was significantly higher in non-rejected recipients. In agreement with our results, previous studies showed that low exposure to cyclosporin was an important risk factor for graft rejection and also late graft loss. Previous studies reported in liver transplant recipients who had cyclosporin concentrations below about 180 ug/l were more likely to suffer rejection [26]. Studies in heart and heart-lung transplantation, has been suggested that the threshold concentrations below which rejection is more likely are 375 ug/l and 500 ug/l respectively [27]. Haematological and biochemical markers were contributed to variability in C0. The statistical strength from these variables was frequently high in the rejected kidney transplant recipients. The association between elevated levels of serum potassium and creatinine perhaps equating with renal dysfunction. It can be argued that renal impairment was present in many patients around the time of rejection. However it is also possible that renal impairment may have resulted (at least in part) from cyclosporin. While there is a precedent for an association between elevated cyclosporin levels and nephrotoxicity [12], the converse effect of renal dysfunction on cyclosporin renal clearance cannot be excluded. Kidney damage may impair the activity of renal CYP450A and may make a significant contribution to total cyclosporin clearance after kidney

transplantation. It is also possible that cyclosporin metabolite accumulates in such circumstances and cross-react with the radioimmunoassay in some patients [4, 5].

Finally, the result of this study illustrates that for significant improvement after kidney transplantation in Isfahan/Iran laboratory drug monitoring in relation to clinical monitoring seems to be essential.

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